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1. Subject of these Test Guidelines

1.1 These Test Guidelines apply to all varieties of *Solanum lycopersicum* L. x *Solanum habroichaites* S. Knapp & D.M. Spooner, *Solanum lycopersicum* L. x *Solanum peruvianum* L. (Mill.) and *Solanum lycopersicum* L. x *Solanum cheesmaniae* (L. Ridley) Fosberg. Such varieties are generally used as rootstocks for tomato varieties (varieties of *Solanum lycopersicum* L. (*Lycopersicum esculentum* L. (Mill.))).

1.2 Rootstocks belonging to *Solanum lycopersicum* L. (*Lycopersicum esculentum* Mill.) or to *Solanum lycopersicum* L. x *Solanum pimpinellifolium* L. (*Lycopersicum esculentum* Mill. x *Lycopersicum pimpinellifolium* Mill.) should be covered by UPOV Test Guidelines TG/44.

2. Material Required

2.1 The competent authorities decide on the quantity and quality of the plant material required for testing the variety and when and where it is to be delivered. Applicants submitting material from a State other than that in which the testing takes place must ensure that all customs formalities and phytosanitary requirements are complied with.

2.2 The material is to be supplied in the form of seed.

2.3 The minimum quantity of plant material, to be supplied by the applicant, should be:

10 g or 2,500 seeds.

In the case of seed, the seed should meet the minimum requirements for germination, species and analytical purity, health and moisture content, specified by the competent authority.

2.4 The plant material supplied should be visibly healthy, not lacking in vigor, nor affected by any important pest or disease.

2.5 The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If it has been treated, full details of the treatment must be given.

3. Method of Examination

3.1 *Number of Growing Cycles*

The minimum duration of tests should normally be two independent growing cycles.

3.2 *Testing Place*

Tests are normally conducted at one place. In the case of tests conducted at more than one place, guidance is provided in TGP/9 "Examining Distinctness".

3.3 *Conditions for Conducting the Examination*

The tests should be carried out under conditions ensuring satisfactory growth for the expression of the relevant characteristics of the variety and for the conduct of the examination.

3.4 *Test Design*

3.4.1 Each test should be designed to result in a total of at least 20 plants, which should be divided between at least two replicates.

3.4.2 When resistance characteristics are used for assessing distinctness, uniformity and stability, records must be taken under conditions of controlled infection and, unless otherwise specified, on at least 20 plants.

3.4.3 The design of the tests should be such that plants or parts of plants may be removed for measurement or counting without prejudice to the observations which must be made up to the end of the growing cycle.

3.5 Additional Tests

Additional tests, for examining relevant characteristics, may be established.

4. Assessment of Distinctness, Uniformity and Stability

4.1 Distinctness

4.1.1 General Recommendations

It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding distinctness. However, the following points are provided for elaboration or emphasis in these Test Guidelines.

4.1.2 Consistent Differences

The differences observed between varieties may be so clear that more than one growing cycle is not necessary. In addition, in some circumstances, the influence of the environment is not such that more than a single growing cycle is required to provide assurance that the differences observed between varieties are sufficiently consistent. One means of ensuring that a difference in a characteristic, observed in a growing trial, is sufficiently consistent is to examine the characteristic in at least two independent growing cycles.

4.1.3 Clear Differences

Determining whether a difference between two varieties is clear depends on many factors, and should consider, in particular, the type of expression of the characteristic being examined, i.e. whether it is expressed in a qualitative, quantitative, or pseudo-qualitative manner. Therefore, it is important that users of these Test Guidelines are familiar with the recommendations contained in the General Introduction prior to making decisions regarding distinctness.

4.1.4 Number of Plants / Parts of Plants to be Examined

Unless otherwise indicated, for the purposes of distinctness, all observations on single plants should be made on 10 plants or parts taken from each of 10 plants and any other observations made on all plants in the test disregarding any off-type plants.

4.1.5 Method of Observation

The recommended method of observing the characteristic for the purposes of distinctness is indicated by the following key in the second column of the Table of Characteristics (see document TGP/9 "Examining Distinctness", Section 4 "Observation of characteristics"):

- MG: single measurement of a group of plants or parts of plants
- MS: measurement of a number of individual plants or parts of plants
- VG: visual assessment by a single observation of a group of plants or parts of plants
- VS: visual assessment by observation of individual plants or parts of plants

Type of observation: visual (V) or measurement (M)

"Visual" observation (V) is an observation made on the basis of the expert's judgment. For the purposes of this document, "visual" observation refers to the sensory observations of the experts and, therefore, also includes smell, taste and touch. Visual observation includes observations where the expert uses reference points (e.g. diagrams, example varieties, side-by-side comparison) or non-linear charts (e.g. color charts). Measurement (M) is an objective observation against a calibrated, linear scale e.g. using a ruler, weighing scales, colorimeter, dates, counts, etc.

Type of record: for a group of plants (G) or for single, individual plants (S)

For the purposes of distinctness, observations may be recorded as a single record for a group of plants or parts of plants (G), or may be recorded as records for a number of single, individual plants or parts of plants (S). In most cases, "G" provides a single record per variety and it is not possible or necessary to apply statistical methods in a plant-by-plant analysis for the assessment of distinctness.

In cases where more than one method of observing the characteristic is indicated in the Table of Characteristics (e.g. VG/MG), guidance on selecting an appropriate method is provided in document TGP/9, Section 4.2.

4.2 Uniformity

4.2.1 It is of particular importance for users of these Test Guidelines to consult the General Introduction prior to making decisions regarding uniformity. However, the following points are provided for elaboration or emphasis in these Test Guidelines:

4.2.2 For the assessment of uniformity, a population standard of 1% and an acceptance probability of at least 95% should be applied. In the case of a sample size of 20 plants, 1 off-type is allowed.

4.3 Stability

4.3.1 In practice, it is not usual to perform tests of stability that produce results as certain as those of the testing of distinctness and uniformity. However, experience has demonstrated that, for many types of variety, when a variety has been shown to be uniform, it can also be considered to be stable.

4.3.2 Where appropriate, or in cases of doubt, stability may be further examined by testing a new seed or plant stock to ensure that it exhibits the same characteristics as those shown by the initial material supplied.

5. Grouping of Varieties and Organization of the Growing Trial

5.1 The selection of varieties of common knowledge to be grown in the trial with the candidate varieties and the way in which these varieties are divided into groups to facilitate the assessment of distinctness are aided by the use of grouping characteristics.

5.2 Grouping characteristics are those in which the documented states of expression, even where produced at different locations, can be used, either individually or in combination with other such characteristics: (a) to select varieties of common knowledge that can be excluded from the growing trial used for examination of distinctness; and (b) to organize the growing trial so that similar varieties are grouped together.

5.3 The following have been agreed as useful grouping characteristics:

- (a) Fruit: green shoulder (characteristic 12)
- (b) Autonecrosis (characteristic 21)
- (c) Resistance to *Meloidogyne incognita* (characteristic 22)
- (d) Resistance to *Verticillium* sp. – Race 0 (characteristic 23)
- (e) Resistance to *Fusarium oxysporum* f. sp. *lycopersici* – Race 0 (ex 1) (characteristic 24.1)
- (f) Resistance to *Fusarium oxysporum* f. sp. *lycopersici* – Race 1 (ex 2) (characteristic 24.2)
- (g) Resistance to *Fusarium oxysporum* f. sp. *lycopersici* – Race 2 (ex 3) (characteristic 24.3)
- (h) Resistance to *Pyrenophaeta lycopersici* (characteristic 28)

5.4 Guidance for the use of grouping characteristics, in the process of examining distinctness, is provided through the General Introduction and document TGP/9 "Examining Distinctness".

6.5 *Legend*

- (*) Asterisked characteristic – see Chapter 6.1.2
- QL Qualitative characteristic – see Chapter 6.3
- QN Quantitative characteristic – see Chapter 6.3
- PQ Pseudo-qualitative characteristic – see Chapter 6.3
- MG, MS, VG, VS – see Chapter 4.1.5
- (a)-(c) See Explanations on the Table of Characteristics in Chapter 8.1.
- (+) See Explanations on the Table of Characteristics in Chapter 8.2.

		English	français	deutsch	español	Example Varieties	
						Exemples	Note/ Nota
						Beispielssorten	
31.	VG (+)	Resistance to Tomato spotted wilt virus (TSWV)	Résistance au virus de la tache bronzée de la tomate (TSWV)	Resistenz gegen das gefleckte Tomaten-bronzenfleckenvirus (TSWV)	Resistencia al virus del bronceado de tomate (TSWV)		
	QL	absent	absente	fehlend	ausente	Big Power	1
		present	présente	vorhanden	presente	Enpower	9
32.	VG (+)	Resistance to <i>Oidium neolyopersici</i> (On)	Résistance à <i>Oidium neolyopersici</i> (On)	Resistenz gegen <i>Oidium neolyopersici</i> (On)	Resistencia a <i>Oidium neolyopersici</i> (On)		
	QL	absent	absente	fehlend	ausente		1
		present	présente	vorhanden	presente	Multifort	9

8. Explanations on the Table of Characteristics

8.1 *Explanations covering several characteristics*

Characteristics containing the following key in the second column of the Table of Characteristics should be examined as indicated below:

- (a) Observations on the plant, stem and leaves should be done after a fruit set on at least five trusses and before ripening of the second truss. Observations should be done before deterioration of the leaves.
- (b) Observations on the fruit should be made on mature fruits from the second or higher truss.
- (c) Observations on the green shoulder and meridian stripes of the fruit should be made on the plant before maturity.

8.2 *Explanations for individual characteristics*

Ad. 1: Seedling: anthocyanin coloration of hypocotyl



Ad. 2: Plant: height

To be observed after fruit set on 5 nodes.

Ad. 3: Stem: anthocyanin coloration of upper third

Most of the varieties are classed 1 to 5. Expression of anthocyanin is influenced by day temperature. Under greenhouse conditions, the variation is rather low.

Ad. 4: Stem: length of internode

The mean length of the internodes between the 1st and 4th trusses should be assessed.

Ad. 7: Leaf: size of leaflets

The size of the leaflet should be observed in the middle of the leaf.

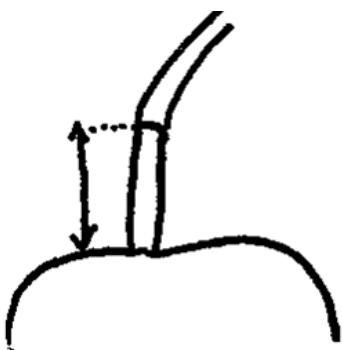
Ad. 9: Leaf: glossiness

The glossiness of the leaf should be observed in the middle of the plant.

Ad. 10: Leaf: blistering

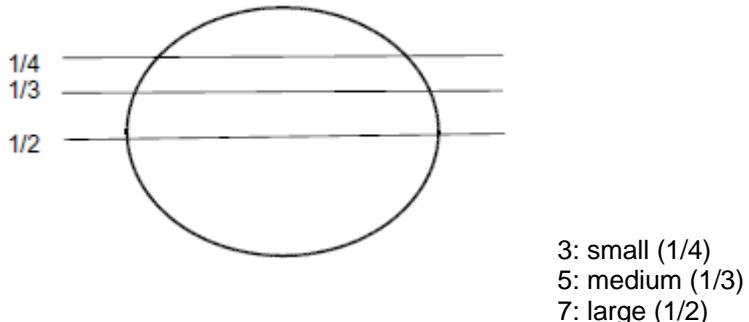
Caution is required for confusion between blistering and creasing. Blistering is the difference in height of the surface of the leaf between the veins. Creasing is independent from the veins. The blistering should be observed in the middle third of the plant.

Ad. 11: Pedicel: length

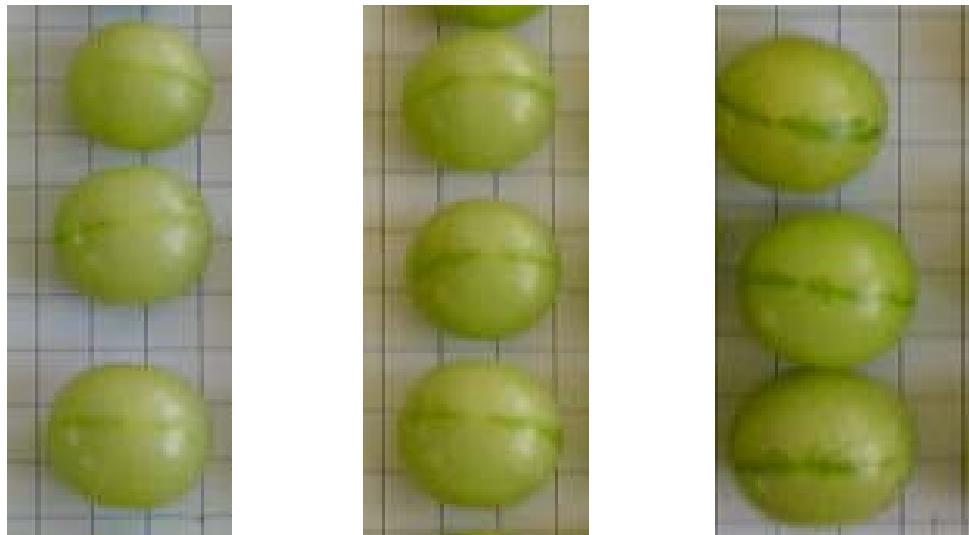


Ad. 13: Fruit: extent of green shoulder

The gene for green shoulder might not be clearly expressed in some conditions.



Ad. 15: Fruit: conspicuousness of meridian stripes



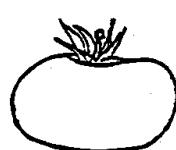
2
weak

3
medium

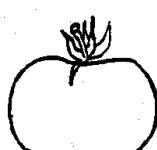
4
strong

Ad. 17: Fruit: shape in longitudinal section

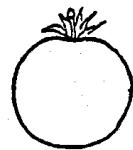
The apex is considered to be the part that is furthest from the stalk attachment.



1
broad oblate



2
narrow oblate



3
circular



4
obovate

Ad. 21: Autonecrosis

Autonecrosis is a necrotic reaction to the presence of incompatible genomes causing older leaves to wither and die.

Ad. 22: Resistance to *Meloidogyne incognita* (Mi)

1. Pathogen *Meloidogyne incognita*
3. Host species *Solanum lycopersicum*
4. Source of inoculum Naktuinbouw (NL¹) or GEVES² (FR)
5. Isolate non-resistance breaking
6. Establishment isolate identity use rootstock or tomato standards
7. Establishment pathogenicity use susceptible rootstock or tomato standard
8. Multiplication inoculum
 8.1 Multiplication medium living plant
 8.2 Multiplication variety preferably resistant to powdery mildew
 8.3 Plant stage at inoculation see 10.3
 8.5 Inoculation method see 10.4
 8.6 Harvest of inoculum root systems are cut with scissors into pieces of about 1 cm length
 8.7 Check of harvested inoculum visual check for presence of root knots
 8.8 Shelf life/viability inoculum 1 day
9. Format of the test
 9.1 Number of plants per genotype 20 plants
 9.2 Number of replicates 1 replicate
 9.3 Control varieties
 Susceptible: Bruce and (*Solanum lycopersicum*) Clairvil, Casaque Rouge
 Moderately resistant : (*Solanum lycopersicum*) Madyta, Campeon, Madyta, Vinchy
 Highly resistant: Emperador and (*Solanum lycopersicum*) "Anahu x Casaque Rouge", Anahu, Anabel
 9.4 Test design include standard varieties
 9.5 Test facility greenhouse or climate room
 9.6 Temperature not over 28°C
 9.7 Light at least 12 h per day
10. Inoculation
 10.1 Preparation inoculum small pieces of diseased root mixed with soil
 mix soil and infested root pieces
 10.2 Quantification inoculum soil: root ratio = 8:1, or depending on experience
 10.3 Plant stage at inoculation seed, or cotyledons
 10.4 Inoculation method plants are sown in infested soil or contamination of soil after sowing when plantlets are at cotyledon stage
 10.7 Final observations 28 to 45 days after inoculation
11. Observations
 11.1 Method root inspection
 11.2 Observation scale Symptoms:
 Galling, root malformation,
 growth reduction, plant death
 11.3 Validation of test evaluation of variety resistance should be calibrated with results of resistant and susceptible controls on standards
12. Interpretation of test results in comparison with control varieties
To consider that resistant varieties may have a few plants with falls. These are not considered as off-types.
Absent (susceptible)..... [1] growth strongly reduced, high gall count
Intermediate
(moderately resistant)..... [2] medium growth reduction, medium gall count
Present (highly resistant)..... [3] no growth reduction, no galls
13. Critical control points: avoid rotting of roots; high temperature causes breakdown of resistance

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² GEVES; Valerie.GRIMAUT@geves.fr

Ad. 23: Resistance to *Verticillium* sp. (Va and Vd)

1. Pathogen *Verticillium dahliae* or *Verticillium albo-atrum* (see note below)
3. Host species *Solanum lycopersicum*
4. Source of inoculum Naktuinbouw³ (NL) and GEVES⁴ (FR)
5. Isolate Race 0 (e.g. strain Toreilles 4-1-4-1)
8. Multiplication inoculum
- 8.1 Multiplication medium Potato Dextrose Agar, Agar Medium "S" of Messiaen
- 8.4 Inoculation medium water (for scraping agar plates) or Czapek Dox broth (3-7 d-old aerated culture at 20-25°C, in darkness)
- 8.6 Harvest of inoculum filter through double muslin cloth
- 8.7 Check of harvested inoculums spore count; adjust to 10⁶ per ml
- 8.8 Shelf life/viability inoculums 1 d at 4°C
9. Format of the test
- 9.1 Number of plants per genotype 35 seeds for 24 plants
- 9.2 Number of replicates 1 replicate
- 9.3 Control varieties
- Susceptible (*Solanum lycopersicum*) Flix, Marmande verte, Clarion, Santonio, Anabel
- Resistant Big Power and (*Solanum lycopersicum*) Monalbo, Elias, Monalbo x Marmande verte, Daniela, Marmande VR
- 9.4 Test design 20 plants inoculated at least, 2 blanks at least
- 9.5 Test facility greenhouse or climate room
- 9.6 Temperature optimal 20-25°C, 20-22°C after inoculation
- 9.7 Light 12 h or longer
10. Inoculation
- 10.1 Preparation inoculums aerated, liquid culture (8.4)
- 10.2 Quantification inoculums count spores, adjust to 10⁶ per ml
- 10.3 Plant stage at inoculation cotyledon to 3rd leaf
- 10.4 Inoculation method roots are immersed for 4 to 15 min in spore suspension.
- 10.7 Final observations 14-33 d after inoculation
11. Observations
- 11.1 Method visual
- 11.2 Observation scale growth retardation, wilting, chlorosis, and vessel browning
- 11.3 Validation of test evaluation of variety resistance should be calibrated with results of resistant and susceptible controls. Standards near borderline R/S will help to compare between laboratories.
12. Interpretation of test results in comparison with control varieties
- absent [1] severe symptoms
- present [9] mild or no symptoms

13. Critical control points:

All symptoms may be present in resistant varieties, but the severity will be distinctly less than in susceptible varieties. Usually resistant varieties will show significantly less growth retardation than susceptible varieties. Observation of vessel browning is important for diagnosis. Usually, vessel browning will not extend to the 1st leaf in resistant varieties. Many hybrid varieties are heterozygous and appear to have mild symptoms in the biotest. Such hybrids are still considered resistant.

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⁴ GEVES; Valerie.GRIMAUT@geves.fr

Ad. 24: Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)

1. Pathogen *Fusarium oxysporum* f. sp. *lycopersici*
3. Host species *Solanum lycopersicum*
4. Source of inoculum Naktuinbouw⁵ (NL) and GEVES⁶ (FR)
5. Isolate Race 0 (ex 1) (e.g. strains Orange 71 or PRI 20698 or Fol 071 1 (ex 2) (e.g. strains 4152 or PRI40698 or RAF 70 and 2 (ex 3)
Individual strains may vary in pathogenicity
6. Establishment isolate identity use differential varieties (see 9.3)
7. Establishment pathogenicity on susceptible tomato varieties
8. Multiplication inoculum
- 8.1 Multiplication medium Potato Dextrose Agar, Medium "S" of Messiaen
- 8.4 Inoculation medium water for scraping agar plates or Czapek-Dox culture medium (7 d-old aerated culture)
- 8.6 Harvest of inoculum filter through double muslin cloth
- 8.7 Check of harvested inoculum spore count; adjust to 10⁶ per ml
- 8.8 Shelf-life/viability inoculum 4-8 h, keep cool to prevent spore germination
9. Format of the test
- 9.1 Number of plants per genotype... at least 20 plants
- 9.2 Number of replicates..... 1 replicate
- 9.3 Control varieties for the test with race 0 (ex 1)
Susceptible (*Solanum lycopersicum*) Marmande, Marmande verte, Resal
- Resistant for race 0 only (*Solanum lycopersicum*) Marporum, Larissa, "Marporum x Marmande verte", Marsol, Anabel
- Resistant for race 0 and 1 (*Solanum lycopersicum*) Motelle, Gourmet, Mohawk
- Control varieties for the test with race 1 (ex 2)
Susceptible (*Solanum lycopersicum*) Marmande verte, Cherry Belle, Roma
- Resistant for race 0 only (*Solanum lycopersicum*) Marporum, Ranco
- Resistant for race 0 and 1 (*Solanum lycopersicum*) Tradiro, Odisea
- Remark: Ranco is slightly less resistant than Tradiro
- Control varieties for the test with race 2 (ex 3)
Susceptible for race 0, 1 and 2..... Emperador
- Resistant for race 0, 1 and 2..... Colosus
- 9.4 Test design >20 plants; e.g. 35 seeds for 24 plants, including 2 blanks
- 9.5 Test facility glasshouse or climate room
- 9.6 Temperature..... 24-28°C (severe test, with mild isolate)
20-24°C (mild test, with severe isolate)
- 9.7 Light..... 12 hours per day or longer
- 9.8 Season all seasons
- 9.9 Special measures slightly acidic peat soil is optimal;
keep soil humid but avoid water stress
10. Inoculation
- 10.1 Preparation inoculum..... aerated Messiaen or PDA or Agar Medium S of Messiaen or Czapek Dox culture or scraping of plates
- 10.2 Quantification inoculum..... spore count, adjust to 10⁶ spores per ml,
Lower concentration for a very aggressive isolate
- 10.3 Plant stage at inoculation..... 10-18 d, cotyledon to first leaf
- 10.4 Inoculation method..... roots and hypocotyls are immersed in spore suspension
for 5-15 min; trimming of roots is an option
- 10.7 Final observations 14-21 days after inoculation
11. Observations
- 11.1 Method visual
- 11.2 Observation scale Symptoms:
growth retardation, wilting, yellowing,
vessel browning extending above cotyledon
- 11.3 Validation of test evaluation of variety resistance should be calibrated with results of
resistant and susceptible controls
12. Interpretation of test results in comparison with control varieties
absent [1] severe symptoms
present [9] mild or no symptoms
13. Critical control points:
Test results may vary slightly in inoculum pressure due to differences in isolate, spore concentration, soil humidity and temperature. Standards near borderline R/S will help to compare between labs.

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⁶ GEVES; Valerie.GRIMAUT@geves.fr

Ad. 25: Resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl.)

1. Pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici*
3. Host species *Solanum lycopersicum*
4. Source of inoculum Naktuinbouw⁷ (NL) and GEVES⁸ (FR)
5. Isolate -
7. Establishment pathogenicity symptoms on susceptible tomato
8. Multiplication inoculum
8.1 Multiplication medium Potato Dextrose Agar or Medium agar "S" of Messiaen
8.4 Inoculation medium water for scraping agar plates or
Czapek-Dox (7 d-old aerated culture)
filter through double muslin cloth
8.6 Harvest of inoculum spore count; adjust to 10^6 per ml
8.7 Check of harvested inoculum 4-8 h, keep cool to prevent spore germination
9. Format of the test
9.1 Number of plants per genotype at least 20 plants
9.2 Number of replicates 1 replicate
9.3 Control varieties
Susceptible: Kemerit and (*Solanum lycopersicum*) Motelle, Moneymaker
Resistant: Emperador and (*Solanum lycopersicum*) Momor, "Momor x
Motelle"
Remark: "Momor x Motelle" has slightly weaker resistance than Momor
9.4 Test design >20 plants; e.g. 35 seeds for 24 plants, including 2 blanks
9.5 Test facility glasshouse or climate room
9.6 Temperature 24-28°C (severe test, with mild isolate)
17-24°C (mild test, with severe isolate)
9.7 Light at least 12 hours per day
9.8 Season all seasons
9.9 Special measures slightly acidic peat soil is optimal;
keep soil humid but avoid water stress
10. Inoculation
10.1 Preparation inoculum aerated culture or scraping of plates
10.2 Quantification inoculum spore count, adjust to 10^6 spores per ml
10.3 Plant stage at inoculation 12-18 d, cotyledon to third leaf
10.4 Inoculation method roots and hypocotyls are immersed in spore suspension
for 5-15 min
10.7 Final observations 10-21 days after inoculation
11. Observations
11.1 Method visual; a few plants are lifted at the end of the test
11.2 Observation scale Symptoms:
Plant death, Growth retardation caused by root degradation
Root degradation, Necrotic pinpoints and necrotic lesions on stems
11.3 Validation of test evaluation of variety resistance should be calibrated with results of
resistant and susceptible controls
12. Interpretation of test results in comparison with control varieties
absent [1] symptoms
present [9] no symptoms
13. Critical control points: Temperature should never exceed 27°C during the test period;
frequent renewal of races may be needed because of loss of pathogenicity

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⁸ GEVES; Valerie.GRIMAUT@geves.fr

Ad. 27: Resistance to Tomato mosaic virus (ToMV)

1. Pathogen	Tomato mosaic virus
3. Host species	<i>Solanum lycopersicum</i>
4. Source of inoculum.....	Naktuinbouw ¹¹ (NL) or GEVES ¹² (FR)
5. Isolate	Strain 0 (e.g. isolate INRA Avignon 6-5-1-1) 1 and 2
6. Establishment isolate identity	genetically defined tomato standards Mobaci (Tm1), Moperou (Tm2), Momor (Tm2 ²) on susceptible plant
7. Establishment pathogenicity	
8. Multiplication inoculum	
8.1 Multiplication medium	living plant
8.2 Multiplication variety	e.g. Moneymaker, Marmande
8.7 Check of harvested inoculum	option: on <i>Nicotiana tabacum</i> "Xanthi", check lesions after 2 days
8.8 Shelf life/viability inoculum.....	fresh>1 day, desiccated>1year
9. Format of the test	
9.1 Number of plants per genotype	at least 20 plants
9.2 Number of replicates.....	1 replicate
9.3 Control varieties	
Susceptible	(<i>Solanum lycopersicum</i>) Marmande, Monalbo
Resistant for ToMV: 0 and 2	(<i>Solanum lycopersicum</i>) Mobaci
Resistant for ToMV: 0 and 1	(<i>Solanum lycopersicum</i>) Moperou
Resistant with necrosis	(<i>Solanum lycopersicum</i>) "Monalbo x Momor"
Resistant	(<i>Solanum lycopersicum</i>) Gourmet
9.4 Test design	blank treatment with PBS and carborundum or similar buffer
9.5 Test facility.....	Glasshouse or climate room
9.6 Temperature	24 to 26°C
9.7 Light	12 hours or longer
9.8 Season.....	symptoms are more pronounced in summer
10. Inoculation	
10.1 Preparation inoculum.....	1 g leaf with symptoms with 10 ml PBS or similar buffer Homogenize, add carborundum to buffer (1 g/30ml)
10.3 Plant stage at inoculation	cotyledons or 2 leaves
10.4 Inoculation method	gentle rubbing
10.7 Final observations	11-21 days after inoculation
11. Observations	
11.1 Method.....	visual
11.2 Observation scale	Symptoms of susceptibility: Mosaic in top, leaf malformation Symptoms of resistance (based on hypersensitivity): Local Necrosis, Top necrosis, Systemic Necrosis
11.3 Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls

Remark: in some heterozygous varieties a variable proportion of plants may have severe systemic necrosis or some necrotic spots while the other plants have no symptoms. This proportion may vary between experiments

12. Interpretation of test results in comparison with control varieties

- absent [1] symptoms of susceptibility
present [9] no symptoms, or symptoms of hypersensitive resistance

13. Critical control points:

Temperature and light may influence the development of necrosis. More light means more necrosis. At temperatures above 26°C the resistance may break down.

Resistant heterozygous varieties may have symptomless plants and plants with severe necrosis; in spite of apparent segregation the sample may be evaluated as uniform for resistance.

Remark Strain INRA Avignon 6-5-1-1 is recommended for ToMV: 0. This strain causes a striking yellow Aucuba mosaic

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Ad. 28: Resistance to *Pyrenopeziza lycopersici* (Pl)

1. Pathogen *Pyrenopeziza lycopersici*
3. Host species *Solanum lycopersicum*
4. Source of inoculum -
5. Isolate -
7. Establishment pathogenicity biotest
8. Multiplication inoculum
8.1 Multiplication medium V8 Agar
8.2 Multiplication variety susceptible tomato variety
8.3 Plant stage at inoculation seed
8.4 Inoculation medium mixture of soil, e.g. (70%), sand (20%) and inoculum (10.1) (10%) or soil mixed with diseased roots cut to small pieces
8.5 Inoculation method sowing, or transplanting at fruit maturity
8.6 Harvest of inoculum diseased roots are harvested after 2-4 months
8.7 Check of harvested inoculum visual inspection of lesions on roots
8.8 Shelf-life/viability inoculum the fungus will not die quickly, but may lose its pathogenicity within a week after isolation on an agar medium
9. Format of the test
9.1 Number of plants per genotype 20 plants
9.2 Number of replicates 1 replicate
9.3 Control varieties
susceptible: Zaraldo and (*Solanum lycopersicum*) Montfavet H 63.5
resistant: Emperador and (*Solanum lycopersicum*) Kyndia, Moboglan, Pyrella
9.5 Test facility greenhouse or climate cell
9.6 Temperature day 24°C, night 14°C
9.7 Light 12 h minimum
10. Inoculation
10.1 Preparation inoculum e.g. double-autoclaved mixture of soil with 10% oatmeal added
e.g. Incubate for 10-14 d at 20°C with occasional, repeated turning
10.3 Plant stage at inoculation 6 weeks
10.4 Inoculation method transplanting into mixture of soil, sand and inoculum (8.4) or soil mixed with diseased roots cut to small pieces or naturally infected soil
10.7 Final observations 6-8 weeks after transplanting (flowering plant)
11. Observations
11.1 Method visual
11.2 Observation scale Symptoms: brown lesions on roots
11.3 Validation of test evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
12. Interpretation of test results in comparison with control varieties
absent [1] symptoms
present [9] no symptoms
13. Critical control points:
The fungus loses its pathogenicity quickly after isolation on an agar medium. It is advisable to keep the isolate alive on living plants.

Ad. 29: Resistance to *Stemphylium* spp. (Ss)

1. Pathogen *Stemphylium* spp. e.g. *Stemphylium solani* (see note below)
3. Host species *Solanum lycopersicum*
4. Source of inoculum GEVES¹³ (FR)
5. Isolate -
7. Establishment pathogenicity biotest
8. Multiplication inoculum
- 8.1 Multiplication medium PDA (12 hours per day under near-ultraviolet light to induce sporulation) or V8
9. Format of the test
- 9.1 Number of plants per genotype at least 20 plants
- 9.2 Number of replicates 1 replicate
- 9.3 Control varieties
- Susceptible: Big Power and (*Solanum lycopersicum*) Monalbo
- Resistant: Body and (*Solanum lycopersicum*) Motelle, F1 Motelle x Monalbo
- 9.5 Test facility greenhouse or climate cell
- 9.6 Temperature 24°C
- 9.7 Light 12 hours minimum
- 9.9 Special measures incubation in tunnel with 100 % relative humidity or humidity tent closed 5 days after inoculation, after this, 80% until end
10. Inoculation
- 10.1 Preparation inoculum sporulating plates (8.1) are scraped and air-dried overnight
The next day plates are soaked and stirred for 30 min
in a beaker with demineralized water, or sporulating plates are scraped with water with Tween
The spore suspension is sieved through a double layer of muslin.
- 10.2 Quantification inoculum 5.10³ – 10⁵ spores per ml
- 10.3 Plant stage at inoculation 20-22 days (three expanded leaves)
- 10.4 Inoculation method spraying
- 10.7 Final observations 4 -10 days after inoculation
11. Observations
- 11.1 Method visual
- 11.2 Observation scale Symptoms:
necrotic lesions on cotyledons and leaves;
yellowing of leaves
- 11.3 Validation of test evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
12. Interpretation of test results in comparison with control varieties
- absent [1] symptoms (11.2)
- present [9] no symptoms, or less than resistant standard
13. Critical control points: 8.1 and 10.1

Note: Some isolates of *Stemphylium* cannot be classified easily as either *Stemphylium solani* or a related species. These *Stemphylium* isolates may still be useful for identifying resistance to *Stemphylium solani*.

¹³ GEVES; Valerie.GRIMAUT@geves.fr

Ad. 30: Resistance to Tomato yellow leaf curl virus (TYLCV)

1. Pathogen Tomato yellow leaf curl virus (see note below)
2. Quarantine status yes
3. Host species *Solanum lycopersicum*
4. Source of inoculum..... -
5. Isolate -
8. Multiplication inoculum
8.6 Harvest of inoculum..... symptomatic leaves may be stored at -70°C
9. Format of the test
9.1 Number of plants per genotype 20 plants
9.2 Number of replicates..... 1 replicate
9.3 Control varieties
Susceptible: (*Solanum lycopersicum*) Montfavet H 63.5
Resistant: (*Solanum lycopersicum*) TY 20, Anastasia, Mohawk
9.5 Test facility..... field with natural disease pressure
9.9 Special measures prevent spread of white-flies
10. Inoculation
10.3 Plant stage at inoculation 6-12 weeks (adult plants)
10.4 Inoculation method vector (Bemisia white-flies carrying TYLCV)
10.7 Final observations 1-2 months after inoculation
11. Observations
11.1 Method..... visual
11.2 Observation scale Symptoms: leaf yellowing and curling
11.3 Validation of test evaluation of variety resistance should be calibrated with results of
resistant and susceptible controls
12. Interpretation of test results in comparison with control varieties
absent..... [1] severe symptoms
present [9] no or mild symptoms
13. Critical control points:

TYLCV is endemic in many tropical and subtropical areas and has a quarantine status in many countries with a temperate climate. TYLCV is on the EPPO alert list. Some TYLCV resistant varieties may be susceptible to the closely related virus Tomato yellow leaf curl Sardinia virus (TYLCSV).

Ad. 31: Resistance to Tomato spotted wilt virus (TSWV)

1. Pathogen Tomato spotted wilt virus (see note below)
2. Quarantine status yes (see note below)
3. Host species *Solanum lycopersicum*
4. Source of inoculum Naktuinbouw¹⁴ (NL), GEVES¹⁵ (FR)
5. Isolate race 0, preferably a thrips-transmission deficient variant
7. Establishment pathogenicity biotest
8. Multiplication inoculum
6 Harvest of inoculum symptomatic leaves may be stored at -70°C
9. Format of the test
9.1 Number of plants per genotype 20 plants
9.2 Number of replicates 1 replicate
9.3 Control varieties
Susceptible: Big Power and (*Solanum lycopersicum*) Monalbo, Momor,
Montfavet H 63.5
Resistant: Empower and (*Solanum lycopersicum*) Tsunami, Bodar, Mospomor,
Lisboa
9.5 Test facility glasshouse or climatic chamber
9.6 Temperature 20°C
9.7 Light 12 hours or longer
9.9 Special measures prevent or combat thrips
10. Inoculation
10.1 Preparation inoculum press symptomatic leaves in ice-cold buffer
0,01 M PBS, pH 7.4, with 0,01 M sodium sulfite or similar buffer
Option: sieve the leaf sap through double muslin
10.3 Plant stage at inoculation one or two expanded leaves
10.4 Inoculation method mechanical, rubbing with carborundum on cotyledons, inoculum
suspension < 10° C
10.7 Final observations 7-21 days after inoculation
11. Observations
11.1 Method visual
11.2 Observation scale Symptoms: top mosaic, bronzing, various malformations, necrosis
11.3 Validation of test evaluation of variety resistance should be calibrated with results of
resistant and susceptible controls
12. Interpretation of test results in comparison with control varieties
absent [1] symptoms
present [9] no symptoms
13. Critical control points:

TSWV has a quarantine status in some countries. TSWV is transmitted by *Thrips tabaci* and Western flower
thrips (*Frankliniella occidentalis*). Pathotype 0 is defined by its inability to break resistance in tomato varieties
carrying the resistance gene Sw-5.

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¹⁵ GEVES; Valerie.GRIMAUT@geves.fr

Ad. 32: Resistance to *Oidium neolyopersici* (On)

1. Pathogen *Oidium neolyopersici* (Powdery mildew)
3. Host species *Solanum lycopersicum*
4. Source of inoculum..... -
5. Isolate see remark under 13
7. Establishment pathogenicity biotest
8. Multiplication inoculum
8.1 Multiplication medium plant
8.3 Plant stage at inoculation 3 weeks
8.4 Inoculation medium..... water
8.5 Inoculation method see 10.4
8.6 Harvest of inoculum by washing off
8.7 Check of harvested inoculum check for contaminants under microscope
8.8 Shelf-life/viability inoculum 1-2 hours
9. Format of the test
9.1 Number of plants per genotype 20 plants
9.2 Number of replicates..... 1 replicate
9.3 Control varieties.....
Susceptible: (*Solanum lycopersicum*) Momor, Montfavet H 63.5
Resistant tomato: Multifort and (*Solanum lycopersicum*) Atlanta, Romiro, PI-247087
9.5 Test facility..... glasshouse
9.6 Temperature 20°C or 18/24°C
9.7 Light 12 hours
10. Inoculation
10.1 Preparation inoculum..... collect spores in water
10.2 Quantification inoculum 10⁴ conidia/ml
10.3 Plant stage at inoculation 3 weeks
10.4 Inoculation method by spraying on leaves or dredging of leaves
10.7 Final observations 7-18 days after inoculation
11. Observations
11.1 Method..... visual
11.2 Observation scale
0. no sporulation
1. necrotic points and sometimes locally restricted sporulation
2. moderate sporulation
3. abundant sporulation
11.3 Validation of test evaluation of variety resistance should be calibrated with results of
resistant and susceptible controls
12. Interpretation of test results in comparison with control varieties
absent..... [1] Moderate or abundant sporulation
present [9] No or restricted sporulation
13. Critical control points:
Resistance-breaking isolates should be avoided. Resistance to *O. neolyopersici* is usually race-specific.
However, as long as a differential series of tomato genotypes with well defined resistances is lacking, it will
remain hard to conclude that different races of *O. neolyopersici* exist.

9. Literature

Arens P., Mansilla C., Deinum D., Cavellini L., Moretti A., Rolland S., van der Schoot H., Calvache D., Ponz F., Collonnier C., Mathis R., Smilde D., Caranta C., Vosman B., 2010. Development and evaluation of robust molecular markers linked to disease resistance in tomato for distinctness, uniformity and stability testing. Theoretical and applied genetics. 120(3): 655-64

Kjellberg, L., 1973: Sortundersökningar av tomat enligt UPOV, Swedish University of Agricultural Sciences, Research Information Centre, Alnarp Trädgaard 162, SE.

Laterrot, H., 1990: Situation de la lutte génétique contre les parasites de la Tomate dans les pays méditerranéens, P.H.M. Revue Horticole, No. 303, January 1990.

International Seed Federation (ISF): Plant Diseases and Resistance
(http://www.worldseed.org/isf/diseases_resistance.html)

10. Technical Questionnaire

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
		Application date: (not to be filled in by the applicant)
TECHNICAL QUESTIONNAIRE to be completed in connection with an application for plant breeders' rights		
1. Subject of the Technical Questionnaire		
Tomato Rootstocks belonging to:		
1.1 Botanical name	<i>Solanum lycopersicum</i> L. x <i>Solanum habrochaites</i> S. Knapp & D.M. Spooner [...]	
1.2 Botanical name	<i>Solanum lycopersicum</i> L. x <i>Solanum peruvianum</i> (L.) Mill. [...]	
1.3 Botanical name	<i>Solanum lycopersicum</i> L. x <i>Solanum cheesmaniae</i> (L.) Ridley Fosberg [...]	
.		
2. Applicant		
Name		
Address		
Telephone No.		
Fax No.		
E-mail address		
Breeder (if different from applicant)		
3. Proposed denomination and breeder's reference		
Proposed denomination (if available)		
Breeder's reference		

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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#4. Information on the breeding scheme and propagation of the variety

4.1 Breeding scheme

- (i) Inbred line []
- (ii) Hybrid []
- (iii) Open-pollinated variety []
- (iv) Other (please provide details) []

Variety resulting from:

4.1.1 Crossing

- (a) controlled cross (please state parent varieties) []

(.....) x (.....)
female parent male parent

- (b) partially known cross (please state known parent variety(ies)) []

(.....) x (.....)
female parent male parent

- (c) unknown cross []

4.1.2 Mutation (please state parent variety) []

4.1.3 Discovery and development (please state where and when discovered and how developed) []

4.1.4 Other (please provide details) []

TECHNICAL QUESTIONNAIRE

Page {x} of {y}

Reference Number:

4.2 Method of propagating the variety

4.2.1 Seed-propagated varieties

- (a) Self-pollination []
 - (b) Cross-pollination
 - (i) population []
 - (ii) synthetic variety []
 - (c) Hybrid []
 - (d) Other []
- (please provide details)

4.2.2 Vegetatively propagated varieties

- (a) cuttings []
- (b) *in vitro* propagation []
- (c) other (state method) []

4.2.3 Other []
(please provide details)

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
5. Characteristics of the variety to be indicated (the number in brackets refers to the corresponding characteristic in Test Guidelines; please mark the note which best corresponds).		
Characteristics	Example Varieties	Note
5.1 Fruit: green shoulder (12)		
absent		1[]
present	Big Force, Maxifort	9[]
5.2 Fruit: shape in longitudinal section (17)		
broad oblate	He-Wolf	1[]
narrow oblate	Gladiator	2[]
circular	Maxifort	3[]
obovate		4[]
5.3 Fruit: number of locules (18)		
only two	Maxifort	1[]
two and three		2[]
5.4 Fruit: color at maturity (19)		
green	Big Force	1[]
yellowish	Vigomax	2[]
orangish	Titron	3[]
reddish	Brigeor	4[]
5.5 Resistance to <i>Meloidogyne incognita</i> (Mi) (22)		
susceptible	Bruce	1[]
moderately resistant		2[]
highly resistant	Emperador	3[]
5.6 Resistance to <i>Verticillium</i> sp. (Va and Vd) - Race 0 (23)		
absent		1[]
present	Big Power	9[]

TECHNICAL QUESTIONNAIRE		Page {x} of {y}	Reference Number:
Characteristics		Example Varieties	Note
5.7	Resistance to <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (Fol) (24)		
5.8	Race 0 (ex 1) (24.1)		
	absent		1[]
	present	Emperador	9[]
5.9	Race 1 (ex 2) (24.2)		
	absent		1[]
	present	Emperador	9[]
5.10	Race 2 (ex 3) (24.3)		
	absent	Emperador	1[]
	present	Colosus	9[]
5.11	Resistance to <i>Fusarium oxysporum</i> f. sp. <i>radicis-lycopersici</i> (Forl) (25)		
	absent	Kemerit	1[]
	present	Emperador	9[]
5.12	Resistance to <i>Pyrenopeziza lycopersici</i> (Pl) (28)		
	absent	Zaraldo	1[]
	present	Emperador	9[]

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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6. Similar varieties and differences from these varieties

Please use the following table and box for comments to provide information on how your candidate variety differs from the variety (or varieties) which, to the best of your knowledge, is (or are) most similar. This information may help the examination authority to conduct its examination of distinctness in a more efficient way.

Denomination(s) of variety(ies) similar to your candidate variety	Characteristic(s) in which your candidate variety differs from the similar variety(ies)	Describe the expression of the characteristic(s) for the similar variety(ies)	Describe the expression of the characteristic(s) for your candidate variety
<i>Example</i>	<i>Fruit: green shoulder</i>	<i>present</i>	<i>absent</i>
Comments:			

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
<p>#7. Additional information which may help in the examination of the variety</p> <p>7.1 In addition to the information provided in sections 5 and 6, are there any additional characteristics which may help to distinguish the variety?</p> <p>Yes [] No []</p> <p>(If yes, please provide details)</p> <p>7.2 Are there any special conditions for growing the variety or conducting the examination?</p> <p>Yes [] No []</p> <p>(If yes, please provide details)</p> <p>7.3 Other information</p>		
<p>8. Authorization for release</p> <p>(a) Does the variety require prior authorization for release under legislation concerning the protection of the environment, human and animal health?</p> <p>Yes [] No []</p> <p>(b) Has such authorization been obtained?</p> <p>Yes [] No []</p> <p>If the answer to (b) is yes, please attach a copy of the authorization.</p>		

* Authorities may allow certain of this information to be provided in a confidential section of the Technical Questionnaire.

TECHNICAL QUESTIONNAIRE	Page {x} of {y}	Reference Number:
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9. Information on plant material to be examined or submitted for examination.

9.1 The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc.

9.2 The plant material should not have undergone any treatment which would affect the expression of the characteristics of the variety, unless the competent authorities allow or request such treatment. If the plant material has undergone such treatment, full details of the treatment must be given. In this respect, please indicate below, to the best of your knowledge, if the plant material to be examined has been subjected to:

- | | | |
|---|---------|--------|
| (a) Microorganisms (e.g. virus, bacteria, phytoplasma) | Yes [] | No [] |
| (b) Chemical treatment (e.g. growth retardant, pesticide) | Yes [] | No [] |
| (c) Tissue culture | Yes [] | No [] |
| (d) Other factors | Yes [] | No [] |

Please provide details for where you have indicated "yes".

.....

10. I hereby declare that, to the best of my knowledge, the information provided in this form is correct:

Applicant's name

Signature

Date

[End of document]